BIOTRANSFORMATION OF TEBUCONAZOLE BY MICROORGANISMS: EVIDENCE OF A COMMON MECHANISM

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Abstract. A major problem with organic wood preservatives is biotransformation by both wood decaying and wood-inhabiting but nondecaying microorganisms in long-term service. Detoxification of organic biocides may contribute significantly to treated wood failure. In this study, a bacterium (*Pseudomonas fluorescens*), mold (*Trichoderma harzianum*), soft rot (*Chaetomium globosum*), white rot (*Phanerochaete chrysosporium*), and brown rot (*Meruliporia. incrassata*) were used to access the extent of biotransformation and the initial metabolite products of tebuconazole in liquid cultures. This study proposed metabolic pathway(s) and explored the possibility of a common biotransformation mechanism for all species. *P. chrysosporium* showed little ability to metabolize tebuconazole. Within 21 da, 40.4, 59.9, 68.2, and 70.2% tebuconazole was metabolized by *M. incrassata, C. globosum, T. harzianum,* and *P. fluorescens,* respectively, into a form that may be less toxic. Mass spectroscopy and infrared analysis of isolated metabolites indicated that the major pathway was cleavage of the triazole ring on tebuconazole and that most species mainly performed oxidation reactions to form the alcohol monolog, which was further oxidized to form the carboxylic acid analog of tebuconazole. Only *T. harzianum* metabolized the hydroxyl group on the tert-butyl moiety by acetylation to form an ester.

Keywords: Biotransformation, preservative tolerance, oxidation, tebuconazole, wood decay.

INTRODUCTION

The trend toward the use of environmentally sound building materials has increased the interest in wood preservation based on boron or organic systems. Much attention is being directed toward the formulation and testing of metal-free preservative systems, and organic biocides will undoubtedly play an increasing role in the future (Schultz and Nicholas 2007). The major advantage of organic systems is also their major disadvantage: their biotransformation by nondegrading microorganisms that are wood-inhabiting and degrading. Other concerns with organic systems are poor photoprotection and hydrophobicity (Evans 2003).

Intricate groups of highly diverse microorganisms sequentially colonize wood in aboveground or ground-contact exposure. Any of the microorganisms present, including nonwood-destroying organisms, is capable of biotransformation of chemicals at a faster rate than that observed in laboratory studies (Briscoe et al 1990). Thus, an initial species may modify an otherwise unsuitable environment to be suitable to another microorganism. Microbial-mediated detoxification of biocides may contribute significantly to the failure of treated wood (Briscoe et al 1990).

Bacteria have long been recognized for their ability to cause pronounced increase in wood

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permeability and, more recently, to degrade preservative-treated wood and highly durable timbers containing high extractive levels (eg *Eusideroxylon zwageri*) and timbers of high lignin content (eg *Alstonia scholaris*) (Daniel 2003). Wallace and Dickinson (2004) isolated 11 species of proteobacteria that had ability to degrade 3-benzo[b]thien-2-yl-5, 6-dihydro-1, 4, 2-oxathiazine-4-oxide. Bacteria have caused reductions in biocide concentrations ranging from 14% for 2-thiocyanomethylthio benzothiazole to nearly 65% in methylene-bis-thiocyanate (MBT) (Briscoe et al 1990). Wallace and Dickinson (2006) demonstrated that tebuconazole, chlorothalonil, and 3-Iodo-2-propynyl butylcarbamate (IPBC) were detoxified when exposed to the *Rastolia* bacteria.

Wood-inhabiting but nondecaying fungi are also capable of metabolizing a toxic compound into a less potent derivative, thus rendering it less effective in protecting wood from typical wood-destroying fungi (Duncan and Deverall 1964; Ruddick 1984). Increases in both threshold and toxic limits have been observed when wood treated with alkyl-ammonium compounds are first subjected to staining fungi or molds and then basiodiomycetes (Ruddick 1984). *T. harzianum* is known to be tolerant to the antisapstain MBT at concentrations of 4 – 34 ppm and triazoles at concentrations below 200 ppm (Obanda et al 2008). *Trichoderma* spp. are present in nearly all soils and other diverse habitats. The mold *Gliocladium roseum* is capable of transforming didecyldimethylammonium chloride (DDAC). In one study, nearly 50% of the DDAC in sawdust was degraded over 11 wk to a hydroxylated quaternary–ammonium compound (Dubois and Ruddick 1998). It was hypothesized that the alteration of the quat compounds may detoxify the quat to a level that wood-rotting basidiomycetes could grow. *P. chrysosporium* is recognized for its ability to metabolize a large diversity of compounds, including pentachlorophenol (penta), through its lignin degrading system (Mendoza-Cantu et al 2000). Wood-destroying basidiomycetes that secrete the enzyme laccase into the culture medium are able to overcome the toxic effects of penta (Lyr 1962). *M. incrassata* has been reported to tolerate chromated copper arsenate (CCA) and cause substantial weight loss in pine blocks treated with CCA or copper sulphate (Illman and Highley 1996).

The role of bacteria and fungi in the degradation of organic preservatives is important in the long-term performance of treated wood. Thus, activity against the causal decay organisms and resistance to physical losses are no longer the sole performance criteria that need to be understood when developing new wood preservatives. However, despite the role played by microorganisms in preservative detoxification, they are often not considered or understood when developing new wood biocides. As the wood preservative industry becomes more reliant on wholly organic fungicides, there is a need to establish biotransformation pathways of biocides and use this knowledge to minimize the detoxification process by bacteria, fungi, and termites (Wallace and Dickinson 2006).

In the current study, bacterium (*P. fluorescens* Migula NCCB 82001), mold (*T. harzianum* Rifai Strain T-39), soft rot (*C. globosum* Strain 414), white rot (*P. chrysosporium* BKMF 1767), and brown rot (*M. incrassata* MAD 563) were used to access biotransformations and the initial products of metabolism of tebuconazole. A previous study by Obanda et al (2008) showed that triazole ring cleavage is the major pathway used by *T. harzianum* in metabolizing and tolerating tebuconazole. This study proposes metabolic pathway(s) for the metabolism and explores the possibility of a common biotransformation mechanism for all species.

MATERIALS AND METHODS

Test Chemicals and Fungi

Ninety-five percent tebuconazole (Preventol A8) was provided by Lanxess Corp (Pittsburgh, PA), and the standard (99%) was obtained from Sigma Aldrich (St. Louis, MO). An original freeze-dried strain of the bacteria species (ATCC No 11150) was obtained from the

American Type Culture Collection (Manassas, VA). All fungal species were provided by the USDA Forest Products Laboratory (Madison, WI). Fungal cultures were grown and maintained on media containing 1.5% agar, 2% malt extract, and 0.001% yeast extract.

Biotransformation Studies

The minimal inhibition concentration (MIC) is the lowest concentration at which no visible growth of mycelium can be observed. Preliminary work involved the determination of the MIC for tebuconazole against *P. chrysosporium, M. incrassata, T. harzianum*, and *C. globosum*. Plates of nutrient agar containing tebuconazole in concentrations ranging from zero (control) to 200 ppm were prepared in triplicate and kept at 25°C for 14 da. Subsequent biotransformation studies were performed using concentrations lower than the MIC.

The biotransformation study used nutrient-limited B3 medium for nitrogen limitation. B3 represents more a more applicable situation for wood that has low amounts of nitrogen. B3 media per liter of distilled water contained: 1.73 t-aconitic acid adjusted to 4.5 pH with KOH, 2 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g $CaCl_2 \cdot 2\ H_2O$, 0.2 g ammonium tartrate, 10 g glucose, 0.001 g thiamine, and 10 mL mineral elixir. Mineral elixir contained the following per liter: 1.5 g nitrilotriacetic acid (pH adjusted to 6.5 with KOH), 3.0 g $MgSO_4 \cdot 7\ H_20$, 0.5 g $MnSO_4 \cdot H_2O$, 1.0 g NaCl, 0.1 g $FeSO_4 \cdot 7\ H_2O$, 0.1 g $CoSO_4$, 0.1 g $CaCl_2$, 0.1 g $ZnSO_4 \cdot 7\ H_2O$, 0.01 g $CuSO_4$, 0.01 g $AlK(SO_4)_2 \cdot 12\ H_2O$, 0.01 g H_3BO_3, and 0.01 g $NaMoO_4 \cdot 2\ H_2O$. Because B3 was studied and developed specifically for *P. chrysosporium* extracellular peroxidase production, it was necessary to ascertain its suitability for growing *C. globosum, M. incrassate*, and *T. harzianum* in subsequent studies to a previous study (data not shown).

B3 media were supplemented with 0.1 mL of 0.02% tebuconazole to give a final concentration of 2 ppm for the soft rot, white rot, and brown rot species. For *T. harzianum,* 0.1 mL of 0.2% tebuconazole was used to give a final concentration of 20 ppm. Flasks were inoculated in triplicate with an agar plug from the edge of actively growing colonies, incubated at 25°C, and shaken at 120 rpm for 3 – 21 da. For the bacteria strain, flasks containing 100 mL Luria Bertani (LB) media were supplemented with 0.1 mL of 0.2% tebuconazole to make a 20 ppm solution. An overnight *P. fluorescens* culture (100 μL) was added and flasks incubated at 30°C and shaken at 120 rpm for a total of 21 da. LB contained 10.0 g/L tryptone, 5.0 g/L yeast extract, and 5.0 g/L NaCl. All samples were centrifuged 10 min at 15,000 g and 4°C with a fixed-angle rotor, induction-drive centrifuge (Beckman model J2-21M). Culture controls consisted of media blanks in which microorganisms were grown under identical conditions but without tebuconazole. Substrate control consisted of tebuconazole and sterile media incubated without inoculum to determine if tebuconazole would chemically decompose or transform under experimental conditions.

Quantitative Determination of Tebuconazole Retained in Samples

Five milliliters of filtered medium was extracted in solid-phase extraction (SPE) columns. The columns (Bond Elut –C18, 500 mg, 3 mL Varian #12102028) were preconditioned with two column volumes of methanol followed by two column volumes of milli-Q water. The sample was loaded, pulled through, and retained compounds were eluted with four column volumes of methanol. Each sample was redried on a nitrogen evaporator to 5 mL and cleaned with a 0.2 μm Teflon filter before quantitative analysis by gas chromatography (GC-MS). The GC-MS was an Agilent 6890 gas chromatograph using a 5973 mass selective detector. The injection port temperature was 250°C, detector temperature was 280°C, and the carrier gas was helium at 1 mm/min^{-1}. The oven was initially held at 80°C for 2 min followed by a $30°C/min^{-1}$ increase to 190°C and then an $8°C/min^{-1}$ increase to a final temperature of 300°C. The capillary column (model no. RTX35) was 30 m × 250 μm × 0.25 μm. Injection volume was

2.0 μL and total run time was 20.42 min. Tebuconazole eluted at 15.9 min. Running in selected ion mode, ions detected for tebuconazole were 250 as the target ion and 125 and 252 as the qualifier ions. Calibration was done using a four-point curve. Concentrations were calculated by the formula:

$$C = (X/V1) * (V2) * \text{dilution factor} \quad (1)$$

where:
X = value obtained from the curve,
V_1 = initial vol. of sample extracted by SPE,
V_2 = final volume of sample after SPE, and
C = concentration.

To evaluate tebuconazol adsorbed on mycelia, three flasks for each fungal species were separately filtered after 21 da, freeze-dried, and ground. Mycelia were extracted three times using 100 mL of methanol while shaking at 100 rpm for 24 h. The filtrate was centrifuged (10 min at 15,000 g and 4°C) and the extract subjected to SPE before analysis by GC-MS.

High-Performance Liquid Chromatography Analysis

To isolate metabolites in sufficient quantities for structural elucidation, the biotransformation process was carried out on a larger preparative scale. For each fungal species, 10 Erlenmeyer flasks containing 100 mL of B3 media were supplemented with tebuconazole to give a final concentration of 2 ppm for *C. globosum, P. chrysosporium,* and *M. incrassate* and 20 ppm for *T. harzianum*. Flasks were inoculated with an agar plug from an actively growing colony incubated at 25°C and shaken at 100 rpm for 21 da. For the bacteria, 10 flasks containing 100 mL LB media were supplemented with 0.1 mL of 0.2% tebucanazole to yield 20 ppm. An overnight *P. fluorescens* culture (100 μL) was added and flasks were incubated at 30°C while shaking at 120 rpm for 21 da. Each of the 10 samples was then centrifuged (10 min at 15,000 g and 4°C) and filtered and pooled into two equal composite samples.

Each composite sample was subjected to extraction and cleanup twice using 75 mL of dichloromethane. The bottom layer was passed over sodium sulfate and solvent exchanged with 5 mL of acetonitrile before qualitative analysis by high-pressure liquid chromatography (HPLC). Microorganisms were grown under identical conditions but without tebuconazole. Substrate controls consisted of tebuconazole and each sterile media. Several reverse-phase HPLC runs were done to purify metabolites. The system consisted of a Waters 600 pump with a Waters 2487 ultraviolet detector whose output was connected to a fraction collector. The column was a Waters Delta-Pak C18, 3 PM, 15 μm, 8 mm × 100-mm radial-compression module.

Eluant composition as a function of time was as follows: isocratic from 0 – 5 min at 70% water + 0.1% formic acid + 30% acetonitrile, subsequent linear increases of acetonitrile over 5 – 30 min to 85% acetonitrile, and finally isocratic at 15% water + 0.1% formic acid + 85% acetonitrile over 31 – 35 min. Flow rate was 1 mL/min^{-1} with detection at 224 nm wavelength. Tebuconazole eluted at 28 min. Authentic metabolite standards were not available. Tebuconazole and metabolite peaks were identified by comparison of elution time, molecular mass, and identification of functional groups in 1) tebuconazole standard; 2) media only after exposure to test species; and 3) media containing tebuconazole after exposure to each test species

Mass Spectral and Infrared Characterization

Molecular mass determination was performed by electrospray ionization. The instrument (Agilent 6210 time of flight mass spectrometer) was operated in positive-ion mode and high resolution. Intact molecular mass of a compound is not a unique identifier. However, determination of exact intact molecular mass was useful because a large part of the molecule (tebuconazole) was already known and only minor unknown change(s) needed to be characterized. Molecular mass was measured to within an accuracy of 5 ppm. FTIR transmission spectra were obtained by a Bruker Tensor 27 system

single-beam instrument connected to an optical user software data analysis program (KBr technique).

RESULTS AND DISCUSSION

Minimum Inhibitory Concentrations

Of the concentrations tested, the lowest at which no growth was observed was 20 ppm for *M. incrassata,* 5 ppm for *P. chrysosporium,* 200 ppm for *T. harzianum*, and 180 ppm for *C. globosum*. Mycelia diameter increases on Petri dishes and MICs are shown in Table 1. A previous study (data not shown) confirmed the suitability of B3 media for *C. globosum, M. incrassate*, and *T. harzianum.*

Quantitative Analysis of Degradation of Tebuconazole

Trends in the reduction of tebuconazole in the media are shown in Fig 1.

Within 21 da, 70.2% of the original 20 ppm of tebuconazole was depleted by bacteria from the LB media. In B3 media, 40.4% had been depleted by *M. incrassata,* 4.1% was depleted by *P. chrysosporium,* 68.2% by *T. harzianum* cultures, and 59.9% in *C. globosum* cultures. Actual values of tebuconazole retained are shown in Fig 2.

Although *P. chrysosporium* is recognized for its ability to metabolize a large diversity of compounds, including penta by a lignin-degrading system (Kullman and Matsumura 1996; Sack et al 1997; Mendoza-Cantu et al 2000), it showed little ability to metabolize tebuconazole. The culture controls had less than 5% of the tebuconazole depleted. The average tebuconazole was depleted in controls and *P. chrysosporium* cultures were not significantly different (p = 0.1, df = 5). This is in agreement with Morrell and Velicheti (1994) who showed that *P. chrysosporium* was not able to significantly degrade triazoles.

Table 1. *Diameter increase*[a] *(mm) and minimal inhibition concentrations (MICs) of the fungi tested.*

Tebuconazole (ppm)	*M. incrassata*	*P. chrysosporium*	*T. harzianum*	*C. globosum*
Agar only (control)	76	76	76	76
0 (solvent–acetone)	76	76	76	76
0.5	76	68	76	76
1	66	10	76	76
1.5	44	5	76	76
2	38	4	76	76
2.5	36	2	76	76
5	24	0	76	76
10	18	0	76	76
15	6		70	70
20	0		70	70
25	0		64	64
50			42	42
80			38	38
100			38	30
150			10	5
180			5	0
200			0	0
MIC	20	2.5	200	180

[a] Diameter increase = [(diameter of mycelia – diameter of the original plug (10 mm)].

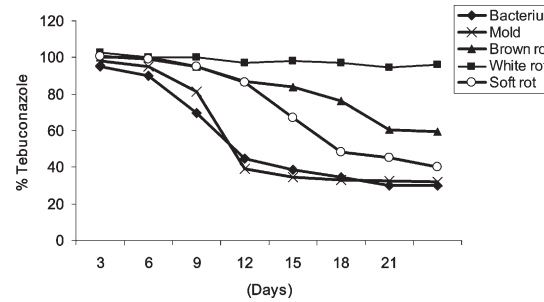

Figure 1. Trends in reduction of tebuconazole in media over 21 da.

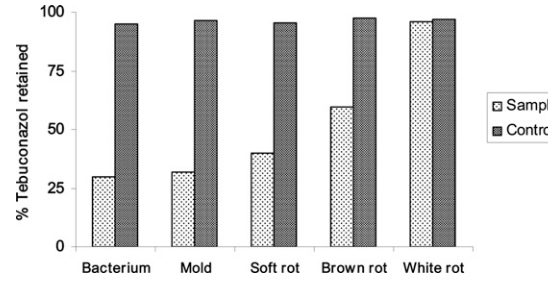

Figure 2. Tebuconazole retained in media after 21 da.

High-Performance Liquid Chromatography Analysis

Secondary metabolites produced by microorganisms did not appear to affect the analysis of tebuconazole. Two major metabolites not found in control cultures were eluted and isolated in cultures of *M. incrassata, C. globosum,* and *P. fluorescens*. Four were isolated in *T. harzianum* cultures. No metabolite was isolated from *P. chrysosporium* cultures. Tebuconazole eluted at 28 min. Major components of B3 media eluted at 2 and 21 min, and major components of LB media eluted at 5 and 21 min. Eluting compounds thought to be metabolites were those not found in the media and eluting at times different from that of tebuconazole during the run. Metabolites in *T. harzianum* cultures had retention times of 10, 13, 15, and 30 min (M1, M2, M3, and M4, respectively), whereas those in *P. fluorescens, M. incrassata,* and *C. globosum* cultures had retention times of 13 and 15 min (M2 and M3, respectively).

Mass Spectral and Infrared Characterizations

The ^{35}Cl:^{37}Cl (3:1) intensity ratio in tebuconazole (mass of 308.15) and in all metabolites (Figs 3 and 4a) was useful to differentiate between degradation products vs molecules arising from the media or fungal secretions (Stoll et al 2006). There is a possibility that metabolites without Cl remained unidentified.

Because the molecular ions were generated by protonation, tebuconazole and three of the biotransformation products showed an even mass, indicating presence of an odd number (three) of nitrogens (reversed nitrogen rule) (McLafferty and Turecek 1993). This observation led to the conclusion that these biotransformation products contained the intact triazole ring. Table 2 presents a summary of dominant ions and intensities. One major product (M3) found in all cultures in which biotransformation occurred had a molecular mass of 281.17 indicating an even number of nitrogens. This and a loss of 27 amu led to the conclusion that the HCN fragment was lost after cleavage of C-N and/or N-N bonds on the 1,2,3-triazole ring to form an imine. Tebuconazole, M1, M2, and M4 all had a degree of unsaturation of 7, whereas M3 has 6 unsaturated bonds, a further confirmation of the cleavage of the triazole ring. Two peaks corresponding to 281.17 were observed in the total ion count (TIC) for M3 (Fig 4b). This corresponds to two possible products of triazole

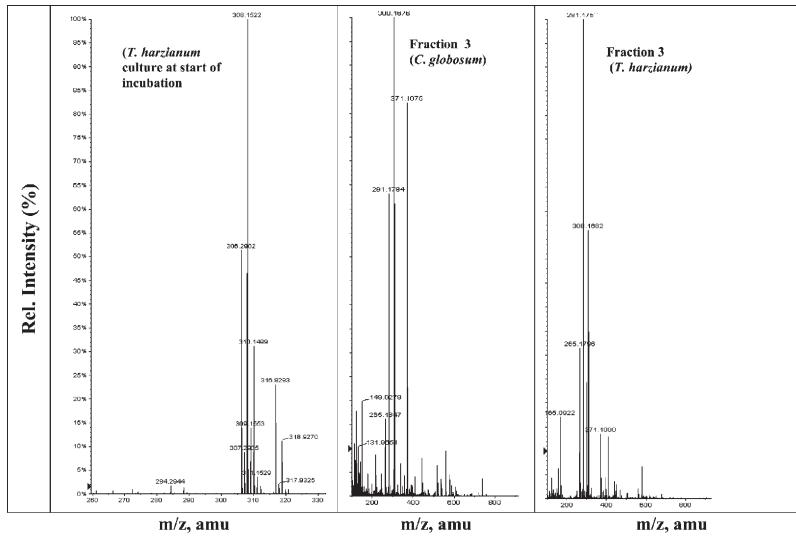

Figure 3. Tebuconazole exact mass and isotope distribution pattern.

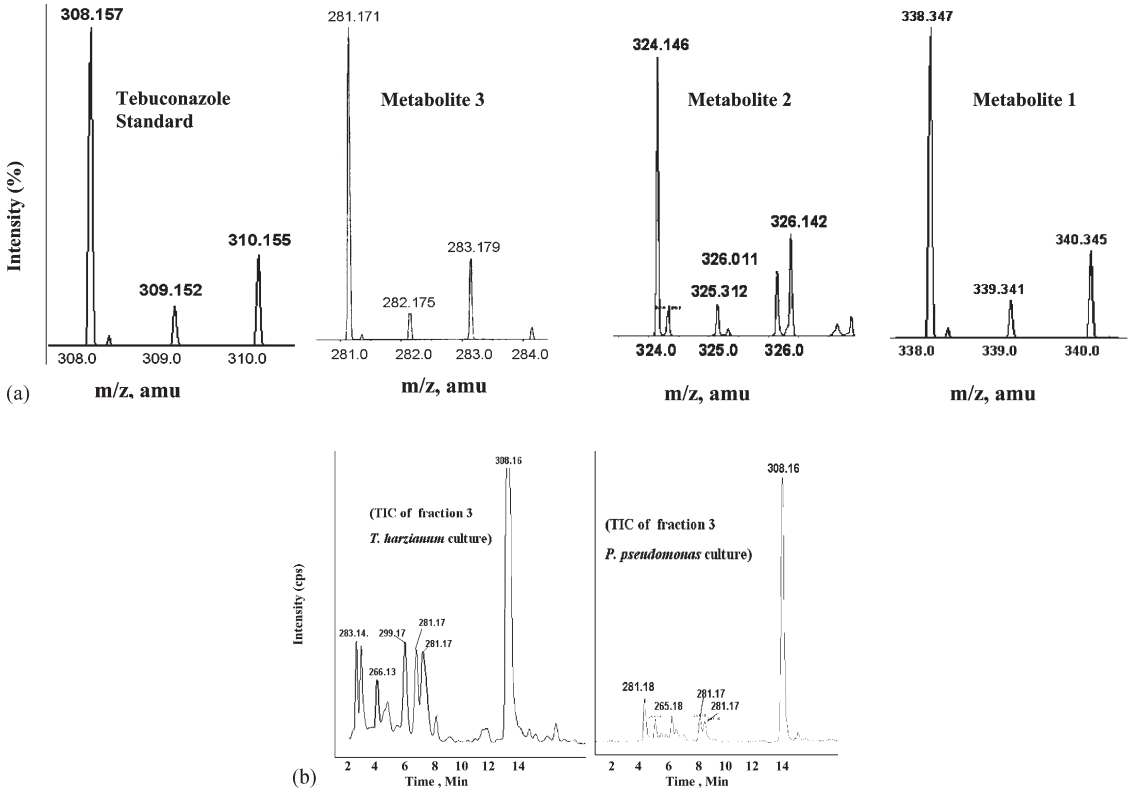

Figure 4. (a) Tebuconazole and metabolite most abundant isotope and isotope distribution pattern. (b) Total Ion Count of fraction 3, which was the most abundant metabolite in all cultures.

cleavage at two different places both resulting in the loss of an HCN fragment. The structures of the two products are shown in Fig 5.

Cleavage of the 1, 2, 4 triazole ring is likely a major pathway for detoxifying tebuconazole by the bacterium and the mold and soft and brown rot fungi used in this study. The action of tebuconazole as a fungicide occurs through binding of the triazole ring to the heme of the cytochrome P-450 enzyme sterol 14α-demethylase and thereby inhibiting ergosterol synthesis in fungi, which in turn retards growth of the fungi. Once the triazole ring is broken down, this mechanism is interfered with and thus compromised.

Establishing the identity of metabolites involved analysis of functional groups in all fractions. Table 3 shows the important infrared spectra bands identified in the fractions of fungal cultures and the bacterial culture. Infrared analysis of functional groups revealed no difference in relevant bands in the spectra of M3 from tebuconazole. Infrared spectra for M1 and M2 showed the presence of carboxylic acid and hydroxyl groups in the molecule. Most likely, these compounds are the primary oxidation products of the methyl groups on the *t*-butyl moiety, ie the hydroxyl and the carboxyl groups readily identified by the sharp C=O stretch between 1680 and 1725 cm^{-1} and O–H stretch of the carboxylic group appearing as a broad peak in the 3000 cm^{-1} region. Oxidation may also have occurred on the phenyl ring and the α-carbon.

All the species initially perform oxidation reactions as a prelude to the detoxification of

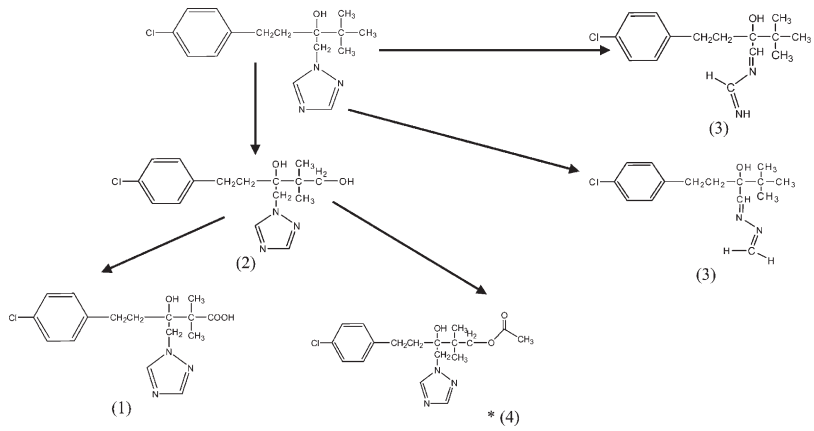

Figure 5. Proposed initial metabolic pathway of tebuconazole (fraction number in parentheses).

tebuconazole as is common with other biocides (Kelley et al 1993; Kanaly and Harayama 2000). In contrast with the results of Strickland et al (2004), there was no evidence of dechlorination or oxidative cleavage of the phenyl ring. Monooxygenase-type reactions occurred in initial oxidation of tebuconazole in agreement with Strickland et al (2004). The oxidation metabolites identified are likely the initial metabolite products. Many fungal species perform phase II reactions after an initial oxidation leading to detoxification of xenobiotics (Cerniglia et al 1982). The mold *T. harzianum* after initial oxidation further metabolizes the hydroxyl group formed on the *t*-butyl moiety by acetylation to form an ester based on the intense strong band in the 1670 – 1700 cm^{-1} double bond region in M4 and a corresponding increase in molecular mass (Table 3). Acetylation deactivates the chemical or its phase 1 metabolite and causes a less hydrophilic product (Cerniglia et al 1982). This step was not observed in the metabolism by *M. incrassata, C. globosum,* and *P. fluorescens*. No evidence of glucosidation and sulphonation phase II reactions was observed.

All the metabolites from *M. incrassata, C. globosum,* and *P. fluorescens* were more hydrophilic than tebuconazole and therefore eluted from the HPLC column faster than tebuconazole. One metabolite from *T. harzianum* was less hydrophilic and eluted after tebuconazole. Because the initial detoxification pathway is the same in all species (oxidation), intervention mechanisms to stabilize or increase the durability of tebuconazole in treated wood would likely involve use of an additive that acts as an inhibitor to the initial oxidation reactions.

The method applied in this study detected only a limited numbers of metabolites. Other probable metabolites could not be identified as a result of low concentrations, fast biotransformation, and interference of media components. Future studies with ^{3}H or ^{14}C-labeled tebuconazole could permit a more complete identification of metabolites and a better proposal of possible biodegradation pathways.

CONCLUSIONS

Although *P. chrysosporium* is recognized for its ability to metabolize a large diversity of compounds, it showed little ability to metabolize tebuconazole. A common mechanism involving the triazole ring cleavage and oxidation reactions on the tert-butyl moiety is apparently the main reactions involved in the biotransformation of tebuconazole by *M. incrassata* (brown rot), *T. harzianum* (mold), *C. globosum* (soft rot), and *P. fluorescens* (bacterium). The main initial products are alcohol, carboxylic acid, and imine after triazole cleavage. Only *T. harzianum* was able to perform phase II-type reactions by

Table 2. *Summary of positive electrospray ionization–mass spectroscopy m/z spectra-dominant ions and intensities.*

Sample		Peaks (m/z, amu)	Intensity counts (%)	Most intense
Tebuconazole standard		308.15	100	308.15
		309.15	10	
		310.15	25	
		371.11	82	
P. fluorescens	Fraction 1	308.15	37	338.34
		281.14	21	
		310.14	13	
		324.15	65	
		338.34	100	
	Fraction 2	281.14	100	281.14
		282.15	25	
		283.14	17	
		308.15	33	
M. incrassata	Fraction 1	308.15	37	324.15
		310.14	21	338.34
		324.15	100	
		338.34	75	
		340.43	18	
	Fraction 2	281.14	100	281.14
		282.15	14	
		283.14	25	
		308.15	23	
P. chrysosporium	No major fraction identified	308.15	100	308.15
		310.15	25	
		338.34	30	
		371.11	80	
B3 media blank		281.19	20	371.21
		284.19	100	121.97
		393.19	17	
		165.09	10	
		265.19	80	
T. harzianum	Fraction 1	308.15	80	338.34
		310.15	25	308.15
		338.34	100	
		340.36	20	
		371.11	82	
		264.13	18	
	Fraction 2	308.15	100	324.15
		310.15	25	308.15
		324.33	80	
		326.32	21	
		312.15	14	
		371.11	75	
	Fraction 3	281.17	100	281.17
		283.18	25	
		308.15	57	
		265.17	33	
		371.10	15	
	Fraction 4	324.15	22	366.37
		308.17	45	
		364.36	21	
		366.37	100	
		368.35	24	
		371.11	72	
C. globosum	Fraction 1	308.15	46	324.43
		310.15	19	338.34
		324.43	100	
		338.34	60	
		340.36	14	
	Fraction 2	281.17	100	281.14
		283.18	25	
		308.15	35	
		265.19	14	
		371.10	17	
Luria bertani media blank		211.15		371.21
		163.15		121.97
		197.13		

Table 3. *Summary of change in molecular mass and relevant infrared (IR) band in all cultures.*

Protonated ion mass	Change in amu	Unique IR band	Proposed pathway
308.15 (tebuconazole)	0	—	
324.15	+16	3200 – 3000	Oxidation
338.34	+30	3500 – 2500 1700 – 1800	1. Oxidation 2. Carboxylation
366.37	+58	1700 – 1800	1. Oxidation 2. Acetylation
281.17	−27	—	Triazole ring cleavage

acetylation. *P. chrysosporium* had the lowest MIC (highest efficacy) and was not able to degrade tebuconazole, whereas *C. globosum* and *T. harzianum* with the highest MIC (lowest efficacy) degraded tebuconazole and tolerated it at concentrations less than 180 ppm and 200 ppm, respectively. Molds such as *T. harzianum,* although not responsible for decay, may lower biocide concentrations or metabolize it into a less potent derivative making the environment suitable to wood-degrading microorganisms.

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